rates: transmission from infected animals to persons clandestinely engaging in ritual slaughter; specifically, an Ethiopian Jewish community. Physicians in countries receiving immigrants should be aware of ceremonial practices that place patients at risk for zoonoses. The severe respiratory manifestations that ensued following aerosol exposure to animal blood or secretions suggest that brucellosis with pulmonary involvement after inhalation of *Brucella*-infected aerosols might be more common than previously documented.

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Follow-up of Ebola Patient, 2014–2015

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To the Editor: The 2014–2015 epidemic of Ebola virus disease (EVD) in West Africa affected 23,666 persons and caused 14,603 deaths (*1*). The World Health Organization (WHO) declared the epidemic a public health emergency (*2*). Although Ebola virus is transmitted by unprotected physical contact with infected persons, published reports about which body fluids are infected or the risk for fomite transmission are few (*3*). For most cases, virus was detected by reverse transcription PCR (RT-PCR) of clinical (saliva, feces, semen, breast milk, tears, nasal blood, skin swab) and environmental specimens (*4*). Earlier reports of the follow-up of recovered patients stated that viral RNA was detected by RT-PCR for up to 33 days in vaginal, rectal, and conjunctival swab samples from 1 patient and up to 101 days in seminal fluid from 4 patients. Infectious virus was detected in 1 seminal fluid sample 82 days after disease onset (*4,5*).

Attendees at the Eighth Meeting of the WHO Advisory Group on the EVD Response (*1*) discussed potential risk factors, including hidden chains of transmission and sexual transmission, and determined the following criteria. A country can declare "interruption of transmission" when 42 days have elapsed since the last diagnosis of a case. A country can declare that the "outbreak has stopped" when test results from the last case are negative twice or after another 90-day interval. For determining a cutoff for finally declaring the strategy and criteria for elimination, extensive follow-up on infectivity of semen in Ebola survivors is needed.

We report follow-up of a man who recovered from EVD and was monitored for 165 days after he was declared Ebola-free. The 26-year-old man from India returned to New Delhi, India, from Liberia on November 10, 2014, with a certificate from the government of Liberia stating that he was "cured" of Ebola. Because EVD is considered an exotic disease in India, he was placed in isolation at the Airport Health Organization quarantine center at Indira Gandhi International Airport, New Delhi (*6*). Serum and semen samples were collected and sent to the National Centre for Disease Control (NCDC), New Delhi, and the National Institute of Virology (NIV), Pune, India. The serum was negative by

Table. Results of attempted real-time RT-PCR and virus isolation from semen of Ebola virus disease survivor, 2014–2015*

Sample no.	Days declared Ebola-free†	RT-PCR C_t		Cell lines used for virus isolation	Passage							
					1		2		3		4	
		NCDC‡	NIV§		CPE	C_t	CPE	C_t	CPE	C_t	CPE	C_t
1	45	17	22.5	Vero CCL81	None	28.5	None	None	None	None	None	None
				Vero E6	None	31.5	None	None	None	None	ND	None
2	64	21	24	Vero CCL81	None	None	None	None	None	None	ND	ND
				Vero E6	None	32.5	None	None	None	None	ND	ND
3¶	77	22	ND	NA	NA	NA	NA	NA	NA	NA	NA	NA
4¶	98	26	ND	NA	NA	NA	NA	NA	NA	NA	NA	NA
5¶	111	27	ND	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	125	30	34	Vero CCL81	None	None	None	None	ND	ND	ND	ND
				Vero E6	None	None	None	None	ND	ND	ND	ND
7	141	28	38	Vero CCL81	None	None	None	None	ND	ND	ND	ND
				Vero E6	None	None	None	None	ND	ND	ND	ND
8	165	28	35.0	Vero CCL81	None	None	None	None	ND	ND	ND	ND
				BHK-21	None	None	None	None	ND	ND	ND	ND

*C_t, cycle threshold; CPE, cytopathic effect; NA, not applicable; NCDC, National Centre for Disease Control, New Delhi, India; ND, not done; NIV, National Institute of Virology, Pune, India; RT-PCR, reverse transcription PCR.
†Primers from http://www.crcf.sn/wp-content/uploads/2014/09/CDC-Ebola-International-Lab.pdf.
‡Primers from the Centers for Disease Control and Prevention, Atlanta, Georgia, USA.
§Primers from (7).
¶Samples received at NCDC only.

real-time RT- PCR ([*7*]; http://www.cdc.gov/vhf/ebola/diagnosis/index.htm) and positive (titer 1:400) by IgG ELISA (*8*). However, the semen was positive for Ebola viral RNA by real-time RT-PCR. A viral RNA extraction kit (QIAGEN, Valencia, CA, USA) was used to extract RNA from samples. Real-time RT-PCRs were set up in a Bio-Rad real-time PCR machine (Model C1000 Touch CFX96; Hercules, CA, USA) by using a SuperScript III Platinum One-Step qRT-PCR kit with ROX (Invitrogen, Carlsbad, CA, USA).

As a confirmatory measure, partial nucleoprotein gene (1216 nt), partial viral protein (VP) gene (337 nt), and the intergenic region near the VP gene along with VP polyA tail (383 nt) from the semen sample were amplified by RT-PCR (*9*). These sequences (GenBank accession nos. KT191140–KT191142) showed 100% similarity with *Zaire ebolavirus* isolate EBOV/DML14077/SLe/WesternUrban/20150630. To avoid cross-contamination, we used a γ-inactivated Ebola Reston virus strain as a positive control. The RT-PCRs were set up in a Bio-Rad thermal cycler (C1000) by using SuperScript III One-Step RT-PCR System with Platinum-Taq-DNA-Polymerase (Invitrogen).

Ebola virus isolation attempts were made from the semen sample in VeroE6 cells, Vero CCL81, *Pipistrellus* bat embryo, and BHK-21 cell lines at NIV. No isolate was obtained. Cycle thresholds of 28.5 and 31.5 were observed in Vero CCL81 and Vero E6 cells, respectively, in the first passage by real time RT-PCR, but no virus or cytopathic effect was detected in the subsequent 2 passages (Table).

According to WHO guidelines, the semen sample was transported from NCDC to a Biosafety Level 4 laboratory at NIV (for virus isolation). At the time of inoculation in cell culture, the sample had been subjected to a single freeze–thaw cycle. The sensitive nature of the virus may be why Ebola virus was not isolated. PCR positivity alone is not sufficient for considering a patient infectious for Ebola; however, because EVD is considered an exotic disease in India, we depended on real-time RT-PCR–based data for establishing EVD positivity.

Follow-up semen samples were positive by real time RT-PCR for up to 165 days after the patient was declared Ebola-free and were negative thereafter. Cycle thresholds of samples tested at NIV were 22.5 on day 45 after being declared Ebola-free, 24 on day 64, 34 on day 125, 38 on day 141, and 35.0 on day 165 (Table).

Clear criteria for elimination and declaration of the end of an outbreak are needed because any misinterpretation or miscommunication among the countries could negatively affect community confidence (*10*). Although we monitored the patient for 165 days, monitoring began ≈10 days after the patient had recovered. Ebola viral RNA persistence has been documented in a human semen sample for up to 10 months after the patient was declared Ebola-free (*11*). According to the data from this study, the current elimination period may need to be extended, and further studies on the infectivity of semen samples from recovered EVD patients are warranted.

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Sustained Elevated Cytokine Levels during Recovery Phase of Mayaro Virus Infection

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To the Editor: Mayaro virus (MAYV), a mosquito-borne alphavirus endemic to South America, causes a self-limiting febrile arthralgia syndrome closely resembling Chikungunya fever (*1*). MAYV has been detected increasingly as imported infections in international travelers returning to Europe and North America (*2–9*). Joint pain, the most prominent symptom, is often long-lasting (several months), sometimes incapacitating (*4*,*6*,*7*,*9*), and may recur (*8*). Arthralgia develops during the acute phase and symmetrically affects the wrists, ankles, and small joints of hands and feet. Joint swelling may occur initially, but permanent joint damage has not been described (*5*). The clinical disease and diagnostic procedures have been described (*1–9*), but immunologic parameters and their possible role in the clinical follow-up of patients (i.e., during the postacute long-lasting arthralgia period) remain to be investigated.

To further our knowledge of MAYV infection, we analyzed cytokine levels in serum samples from 6 travelers to South America who returned to Europe with Mayaro fever (MF). Two of the cases occurred during 2014; 4 occurred during 2011–2013 (*2–5*).

The 6 travelers comprised 2 men and 4 women who were 20–54 (median 36) years of age (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/22/4/15-1502-Techapp1.pdf). The 2 most recent cases occurred in spring 2014 in a 28-year-old female student and a 54-year-old male physician. Serologic testing was performed for both patients at the Bernhard Nocht Institute and confirmed by virus neutralization testing (*4*).

The student had traveled for 3 weeks in Ecuador, visiting rainforest villages and hiking in the jungle. During her